Quantification of Pesticides in Food without Calibration using GC/FID with the Polyarc™ Reactor

Application Note

Pesticides

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Abstract

The quantification of pesticides in food using gas chromatography (GC) is traditionally a time-consuming process that involves the painstaking calibration of detector response for each analyte. The conversion of analytes to methane before their detection with a flame ionization detector (FID) results in a response that is proportional to the number of carbon atoms in the analytes and thereby eliminates the need for detector calibration. In this note, we use this approach and show the application of the Polyarc™ reactor to the quantification of dilute pesticides. The response factors of compounds in a commercial 22-component organochlorine pesticide mixture (200 µg/mL of each component) were 1.00 ± 0.09 with an average deviation from unity of 4%. This is compared to an FID-only analysis with response factors of 0.83 ± 0.10 with an average deviation from unity of 17%. Additional testing of the Polyarc™ reactor for six single-analyte pesticide solutions prepared from pure components provided response factors of 1.00 ± 0.04 with a mean deviation of 2%. Because all compounds have a response factor of one when using the Polyarc™ reactor, calibration to determine response factor is no longer required. We also show that direct-connect splitless liners prevent discrimination of analytes in the GC injector port.

Introduction

Quantification of pesticides and other molecules by GC/FID is often a time-consuming process because the response factors for each analyte must first be determined. Response factors (RF) are typically defined as:

$$RF\ (mol\ \%\ C) = \frac{area_1/_{mol\ C_1}}{area_2/_{mol\ C_2}},$$

where (1) and (2) are the analyte and internal standard, respectively, *area* is the integrated GC/FID peak area (i.e., the integrated detector response), and *mol C* are the injected moles of carbon of the component (the concentration of the component in terms of carbon content in the sample could also be used).

Response factors in GC/FID analyses are dependent on the chemical structure of the molecule. The presence of heteroatoms, such as O, N, P, S, and Cl, change the response of given analyte in an FID detector. The Polyarc™ reactor eliminates the differences in FID response per carbon atom by converting all organic compounds to methane before their detection in the FID:

$$\text{Carbon-Containing Compounds} + \text{Air} + H_2 \rightarrow \text{Methane } (CH_4) + \text{Non-Carbonaceous Byproducts}$$

Since all carbon-containing compounds are converted to methane, the response of the FID is equivalent for all molecules on a per-carbon atom basis.

Experimental

An Agilent 7890A GC equipped with a split/splitless inlet and a Polyarc reactor (ARC PA-RRC-A02) was used for the analysis. Air (zero grade, Praxair) and H_2 (99.999%, Praxair) were supplied to the FID and to the ARC manual flow control module (PA-CAS-A07). Helium (99.999%, Praxair) was used as the carrier gas.

The system was configured with the column connected from the split/splitless inlet to the Polyarc™/FID and to the FID-only. An Agilent G1544-80700 direct connect liner was used, and the inlet was operated in splitless mode.

A commercial pesticide sample (Supelco 8081 pesticide mix, 22 components, 200 µg mL^{-1} in hexane:toluene 50:50) was used without further modification.

GC conditions

Front inlet	Split/Splitless
Inlet temperature	300 ºC
Inlet flow	2.5 sccm He
Septum purge flow	3 sccm (switched)
Oven	40 °C (3 min), 10 °C/min to 130 °C (2 min), 3 °C/min to 280 °C
Column	DB-5, 30 m, 320 µm, 0.25 µm film thickness
Syringe	10 µL
Injection volume	0.1 µL splitless

FID conditions

Temperature	300 °C
H_2	1.5 sccm
Air	350 sccm
Makeup	0 sccm (He)
Sampling rate	50 Hz

Polyarc reactor conditions

Setpoint	293 °C
H_2	35 sccm
Air	2.5 sccm

Results and Discussion

The chromatograms for a 22-component organochlorine pesticide test mixture analyzed with and without the Polyarc™ reactor are shown in Figure 1; molecular structures for the compounds are shown in Figure 2. The full widths at half maximum (FWHM) of the peaks are, on average, 8% greater when using the Polyarc™ reactor in conjunction with the FID. The increased broadening has a negligible influence on the chromatographic separation and all compounds are resolved. The peak positions and shapes are also very similar with and without the Polyarc™ reactor. The peak areas (i.e., a surrogate for sensitivity) obtained with the Polyarc™ reactor are 13% to 43% larger (average of 22%) than those obtained with the FID-only. The elution order was determined with mass spectrometry on a separate GC/MS system. Hexane was used as the internal standard for the analysis. The response factors for the 22 analytes are shown in Figure 3 and Table 1 in order of elution. The response factors for the Polyarc™ reactor are 1.00 ± 0.09 with a mean deviation from unity of 4%. The response factors for the FID-only system have a lower average response of 0.83 ± 0.10 and an increased deviation from unity with an average deviation of 17.1%. The error bars in Figure 3 represent the error obtained from the propagation of the expanded uncertainty in analyte concentration (95% confidence intervals). Within these confidence intervals, the Polyarc™ reactor provides equal response factors (RF = 1) for every organic compound, because all compounds are completely converted to methane before detection in the FID. Thus, calibrations to determine response factor are not necessary when using the Polyarc™ reactor and even the concentration (in carbon amount) of unknown peaks could be determined using RF = 1. Table 1 shows the measured concentrations of the analytes in the mixture using RF = 1 for all components. The average error in concentration is less than 5%, which is within the uncertainty of the measurement.

The minimum detectable limit (MDL) for the Polyarc™ reactor is estimated to be about 100 ng/mL (76 ppb) based on the peak areas obtained in this study and the smallest peak area distinguishable from noise.

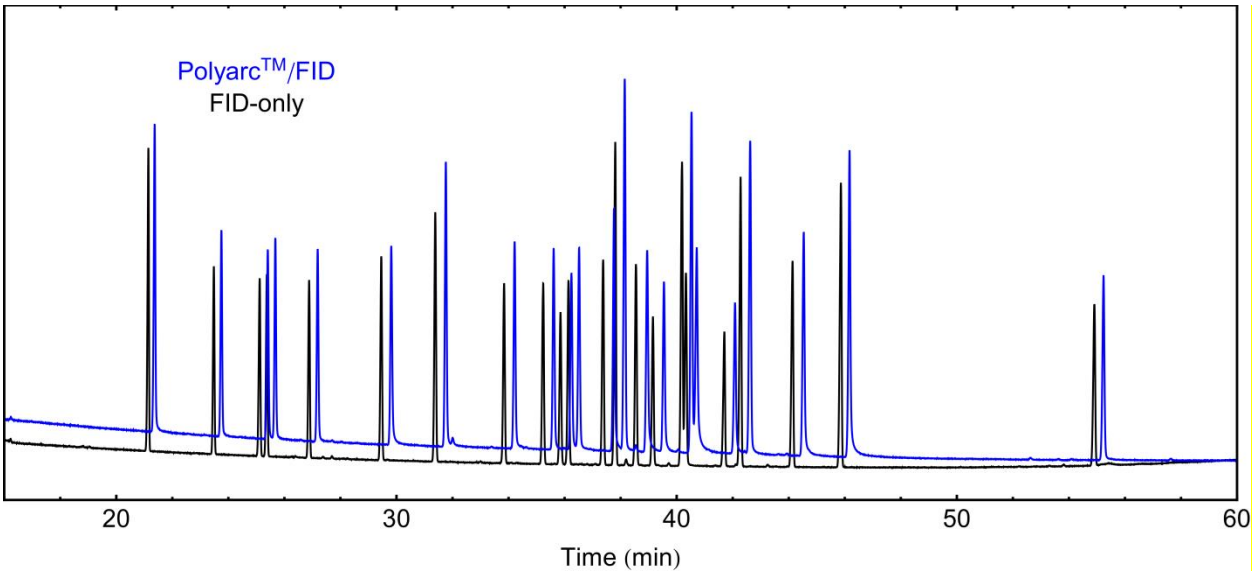

Figure 1. Comparison between the chromatograms obtained for a 22-component organochlorine pesticide test mixture using the Polyarc™/FID and FID-only.

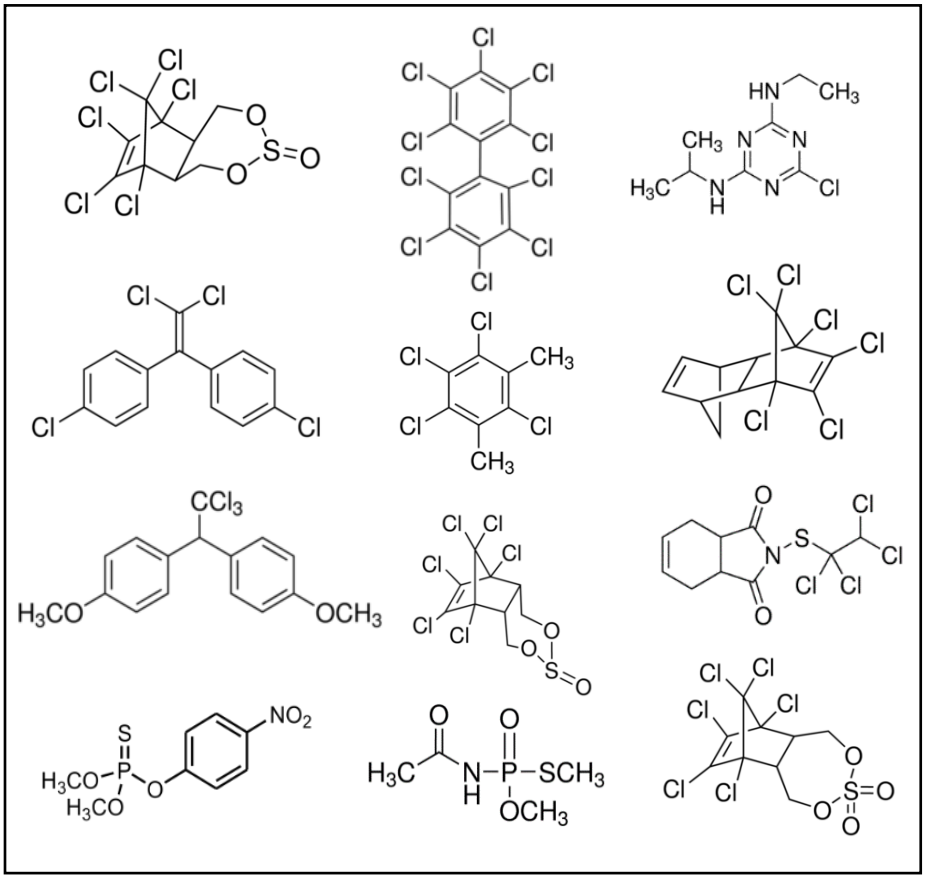

Figure 2. Molecular structures of select compounds analyzed in this study.

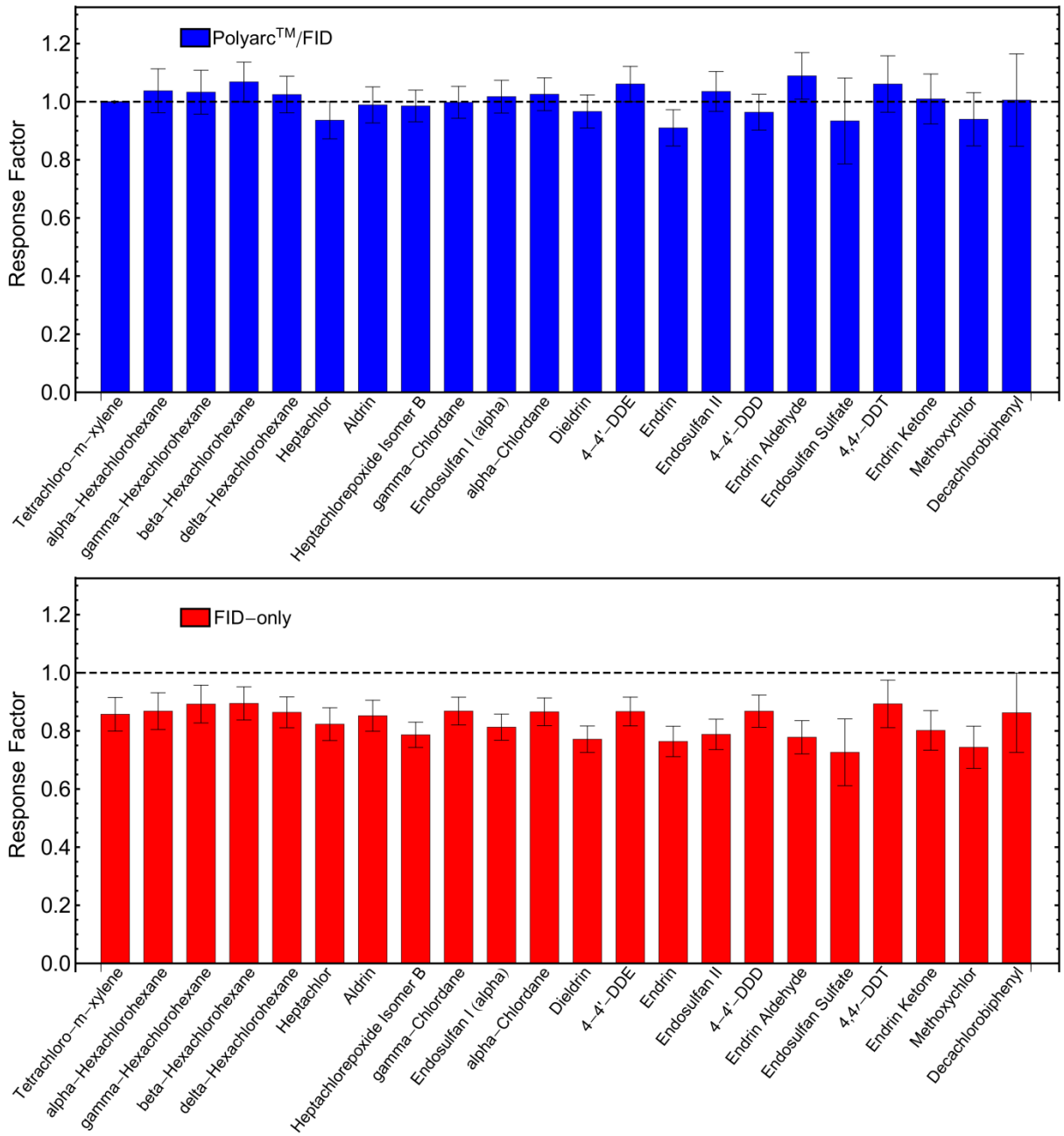

Figure 3. Response factors obtained for a 22-component organochlorine pesticide mixture (200 µg/mL in hexane:toluene 1:1) using the Polyarc™ reactor (top) and FID-only (bottom).

Table 1. Molecular information, peak areas, response factors, and concentrations for the analytes in the 22-component organochlorine pesticide mixture tested with the Polyarc™ reactor.

#	Analyte	Peak Area	Analyte MW (g/mol)	Carbon Number	RF[a]	Expanded Uncertainty	Measured Concentration (µg/mL)[b]	Expanded Uncertainty (µg/mL)
1	Tetrachloro-m-xylene	1583	244.0	8	199	1.00	0.07	200
2	alpha-Hexachlorohexane	1038	290.8	6	200	1.04	0.08	207
3	gamma-Hexachlorohexane	1033	290.8	6	200	1.03	0.08	206
4	beta-Hexachlorohexane	1029	290.8	6	192	1.07	0.07	206
5	delta-Hexachlorohexane	1025	290.8	6	200	1.02	0.06	205
6	Heptachlor	1216	373.3	10	200	0.94	0.06	187
7	Aldrin	1571	364.9	12	199	0.99	0.06	197
8	Heptachloroepoxide Isomer B	1228	389.3	10	200	0.99	0.05	197
9	gamma-Chlordane	1181	409.8	10	200	1.00	0.06	199
10	Endosulfan I (alpha)	1068	406.9	9	196	1.02	0.06	199
11	alpha-Chlordane	1214	409.8	10	200	1.03	0.06	205
12	Dieldrin	1477	380.9	12	200	0.97	0.06	193
13	4-4'-DDE	2233	318.0	14	197	1.06	0.06	209
14	Endrin	1390	380.9	12	200	0.91	0.06	182
15	Endosulfan II	1110	406.9	9	200	1.04	0.07	207
16	4-4'-DDD	1994	320.0	14	195	0.96	0.06	188
17	Endrin Aldehyde	1639	380.9	12	197	1.09	0.08	214
18	Endosulfan Sulfate	997	406.9	9	199	0.93	0.15	186
19	4,4'-DDT	1953	354.5	14	192	1.06	0.10	204
20	Endrin Ketone	1536	380.9	12	199	1.01	0.09	201
21	Methoxychlor	2109	345.7	16	200	0.94	0.09	188
22	Decachlorobiphenyl	1169	498.7	12	199	1.01	0.16	200

[a]Response factors determined using hexane as the internal standard.
[b]Concentrations reported assuming RF = 1 for all analytes.

Additional testing of the Polyarc™ reactor with different molecules and higher concentrations was performed on six solutions of pesticides dissolved in methanol (Figure 4). The analyte concentrations ranged from 1-4 mg/mL. Methanol was used as both the solvent and the internal standard for this analysis. The response factors shown in Figure 4 are 1.00 ± 0.04 with a mean deviation from unity of 0.02. This data provides further confirmation that the Polyarc™ reactor leads to calibration-free analyses as the response factor is equivalent for all molecules.

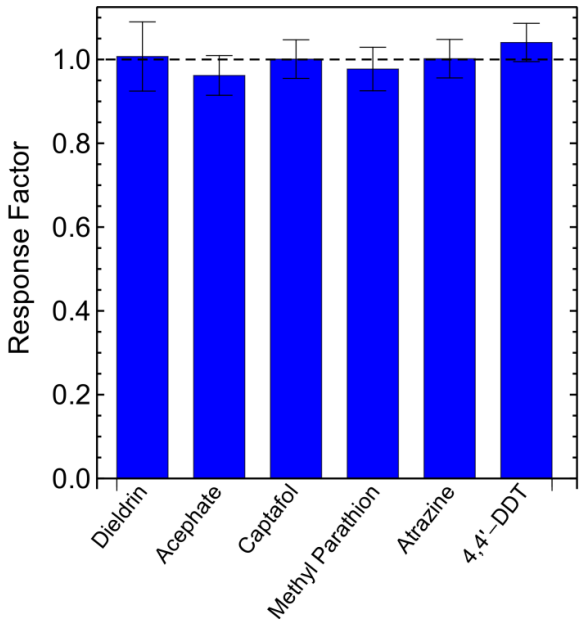

Figure 4. Response factors obtained for six pesticides analyzed with the Polyarc™ reactor

Selecting the correct inlet liner

When performing quantitative GC analyses it is important that inlet conditions are optimized to prevent discrimination of analytes in the GC system. Sources of analyte discrimination include preferential vaporization in the inlet and hold-up. Hold-up is defined as the retention of small amounts of analyte within the inlet, column, or other location of a GC. If analyte discrimination is occurring within an instrument, it will result in inaccuracies, independent of the analysis and detection methods. For this reason, it is very important that discrimination be eliminated using an appropriate instrument setup. For analytes that are prone to discrimination such as high molecular weight compounds or "sticky" compounds with reactive functional groups, it is best to use a deactivated direct-connect splitless liner (e.g., the Agilent G1544-80700 direct connect liner, or the Restek Uniliner®). The direct-connect liners make a sealed connection with the column, preventing preferential vaporization and contact of analytes with metal components within the GC inlet. Furthermore, the inlet liners are deactivated and free of quartz wool to prevent adsorption and subsequent hold-up of the analytes. The data shown above for the 22-component pesticide test mixture was collected with a direct-connect liner in splitless mode. Splitless injections, in general, and direct-connect liners, in particular, require smaller injection volumes to avoid overloading the inlet and column. Peak broadening and fronting can be minimized with the appropriate injection volumes and inlet temperature, although in some cases poor peak shapes are unavoidable.

As an example of poor inlet conditions, the GC was initially equipped with a liner designed primarily for split injections (Agilent part no. 5190-2295) and used in splitless injection mode. The response factors obtained with the Polyarc™ (Figure 5) deviate from unity, demonstrating that absolute injection of all components is not occurring. This is in stark comparison to the results that were obtained using a direct-connect liner (Figure 3) where RF = 1 for all compounds. The low response factor for methoxychlor, for example, indicates that this compound is either retained within the GC inlet or escaping the system through the septum purge or split vent. A response factor greater than one indicates that the compound has a higher injection efficiency than the internal standard, tetrachloro-m-xylene. The discrimination present with the split liner and not the direct-connect liner may be the result of differences in the design of the liners, or adsorption sites present on the split liner from the quartz wool or residual molecules. These results stress the importance of selecting a proper inlet liner when analyzing high molecular weight and/or highly reactive analytes.

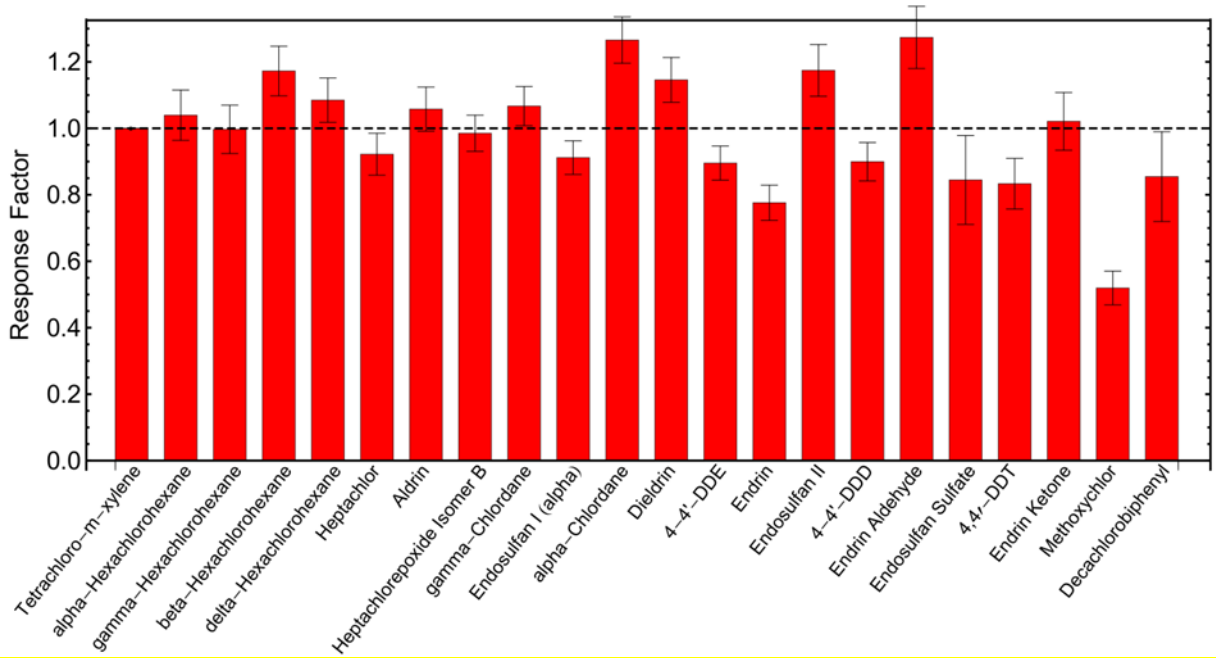

Figure 5. Response factors obtained for a 22-component organochlorine pesticide mixture (200 µg/mL in hexane:toluene 1:1) with a poor absolute injection of analytes onto the column.

Conclusions

The Polyarc™ reactor saves time and makes analysis easier by eliminating the need for calibration in GC/FID analyses of all organic molecules. The ability of the Polyarc™ reactor to perform analysis without calibration is unparalleled in the field of analytical chemistry. Quantification of rare molecules for which standards are prohibitively expensive, or do not exist, is made possible with the Polyarc™ reactor because response factors are unity for all compounds. An additional benefit of the Polyarc™ reactor is its ability to detect and remedy inlet discrimination issues through the analysis of standard component mixtures; if response factors deviate from unity, there may be a problem with the GC (e.g., system leak, discrimination, compound degradation, etc.).

Contact Us

For more information or to purchase a Polyarc® system, please contact us at 612-787-2721.

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